Party #17

PATENT
Attorney Docket No. 4270.0014

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Richard F SELDEN	)	
	)	
Serial No. 08/461,292	)	Group Art Unit: 1804
	)	
Filed: June 5, 1995	)	Examiner: Christopher S. F. Low
	)	
For: TRANSKARYOTIC IMPLANTATION	)	

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

DECLARATION OF HOWARD M. GOODMAN, PH.D. UNDER 37 C.F.R. § 1.132

1. I, Howard M. Goodman, DO HEREBY DECLARE AND SAY:

2. I received an A.B. in physics from Williams College in 1960 and a Ph.D. in biophysics from Massachusetts Institute of Technology in 1964.

3. I am currently the Chief of the Department of Molecular Biology at Massachusetts General Hospital and a Professor in the Department of Genetics at Harvard Medical School.

4. I have held teaching and research positions at medical schools and teaching hospitals in the fields of molecular biology and genetics. These positions are listed in my Curriculum Vitae, which is attached as Exhibit 1.

5. I have published a number of articles in the fields of molecular biology and genetics. Many of these articles concern the molecular biology of genes, the insertion of genes into cells, and the expression of such genes. These articles are also listed in my attached Curriculum Vitae.

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6. I have received several awards and honors in recognition of my work, including the following:

Honorary Doctorate of Science, Williams College, MA (1986);
George W. Thorn Award, Howard Hughes Medical Institute (1981);
H.C. Jacobaeus Foundation Lecturer (1979);
CIBA-GEIGY DREW Award in Biomedical Research (1978);
Research Postdoctoral Fellow, American Cancer Society (1967-1969); and
Research Postdoctoral Fellow, Helen Hay Whitney Foundation (1964-1967).

7. I have been engaged in research relating to the introduction of genes into cells and the expression of genes in cells for over 20 years. My research has involved introducing genes into a variety of animal cells by various methods, and I am well-versed in the literature regarding gene transfer and gene expression methods. These methods include both viral methods (including retroviral methods) to insert a gene into a cell, and nonviral methods, which use a physical or chemical technique to insert a gene into a cell.

8. Prior to 1987, nonviral gene transfer methods had not been used successfully for gene therapy, and those skilled in the art came to the conclusion that such methods were unsuitable for *ex vivo* gene therapy. Those skilled in art thought that *ex vivo* gene transfer for gene therapy required a highly efficient method of introducing a gene into a cell. They believed that the inherent inefficiency of nonviral gene transfer methods presented an obstacle that made their successful use in *ex vivo* gene therapy unlikely. (Ex. 2 at 405, cols. 1 and 3, 406, col. 1; Ex. 3 at 213, col. 2).

9. Rather than nonviral methods, those skilled in the art, at the time of the filing of the Selden application, believed that viral transduction, especially retroviral transduction, was the best method for *ex vivo* gene therapy. (Ex. 2 at 408, col. 1). More efficient retroviral vectors were being developed, and the groups developing these

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vectors and studying retroviral transduction were dominating (and, indeed still dominate) the field of *ex vivo* gene therapy. This belief was so dominant that almost the entire field focused on retroviral transduction to the exclusion of less efficient nonviral methods.

10. I have read and understood U.S. Patent Application Serial No. 07/044,719, entitled "Transkaryotic Implantation" ("the Selden application"). A copy of the Selden application is attached as Exhibit 4. Despite the belief of those skilled in the art that methods using retroviral vectors were the best methods for *ex vivo* gene therapy, and despite their belief that the known problems with retroviral vectors could be overcome, the Selden application states that the use of retroviral vectors "will face major obstacles" and the Selden application discusses several of these "major obstacles":

> In the past few years, it has become apparent that the implementation of retroviral based gene delivery systems in humans will face major obstacles, primarily related to properties of retroviruses themselves (Robertson, M., Nature 320:213-214 (1986), Marx, J.L., Science 232:824-825 (1986)). First, it has not been generally possible to achieve expression of mammalian genes in the retroviral vectors used to infect human cells, and until this problem is solved, the issue of regulated gene expression cannot be addressed. Second, when retroviruses are used to infect marrow cells in batch, essentially every cell is infected, and the site of retroviral integration into the host's genome varies from cell to cell. Since the infected cells are not characterized before reintroduction, the possibility of a deleterious integration event cannot be eliminated. Third, as recombination between the replication-deficient retroviruses utilized for the infection and the endogenous retroviruses present in mammalian genomes is known to occur (Hock, R.A., et al., Nature 320:275-277 (1986)), there is the potential of initiating a chronic retroviral infection in the host animal.

(Ex. 2 at 4, line 16, through 5, line 4).

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11. As can be seen from Figure 1 of the Anderson reference attached as Exhibit 2, a retroviral genome has two parts: 1) the long terminal repeat ("LTR"), including regulatory elements, such as a promoter; and 2) the retroviral structural genes. These elements of retroviral nucleic acid are a source of the problems with retroviral methods that the Selden application discusses.

12. For example, the Selden application states:

> First, it has not been generally possible to achieve expression of mammalian genes in the retroviral vectors used to infect human cells, and until this problem is solved, the issue of regulated gene expression cannot be addressed.

(Ex. 4 at 4, lines 21-25). In other words, at the time of filing of the Selden application, expression of the desired gene had not generally been achieved with retroviral vectors. Retroviral vectors use the retroviral LTRs, which contain promoters that can control the expression of retroviral genes. (Ex. 2 at 406, col. 1). A problem with retroviral promoters for gene delivery was that they result in little, if any, expression of the gene of interest. (Ex. 2 at 406, col. 2; Ex. 3 at 214, col. 1).

13. The Selden application cites the Robertson article (Robertson, 320 Nature 213 (1986)) to support its statement that the problems with retroviral methods are primarily related to properties of the retroviruses themselves. (Ex. 4 at 4, lines 16-21). I have read and understood the Robertson article. A copy of the Robertson article is attached as Exhibit 3.

14. The Robertson article discusses this first problem with retroviral methods that the Selden application discusses:

> [I]t is known that the viral sequences can induce permanent suppression of viral gene expression in embryonic cells, and although the mechanism is unknown it is suspected that the

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> viral LTRs may be responsible. This raises the unwelcome possibility that the failures of stable gene expression in bone marrow are due to analogous mechanisms that cause stem cells to suppress everything under the control of the viral LTR.

(Ex. 3 at 214, col. 1).

15. The Selden application also states:

> Second, when retroviruses are used to infect marrow cells in batch essentially every cell is infected, and the site of retroviral integration into the host genome varies from cell to cell. Since the infected cells are not characterized before introduction, the possibility of a deleterious integration event cannot be eliminated.

(Ex. 4 at 4, lines 25-31). In other words, since DNA of retroviral origin can integrate almost anywhere in a host cell's genome, it can disrupt an important gene or regulatory sequence of the host. (Ex. 2 at 407, col. 2).

16. The Robertson article also discusses this second problem with retroviral methods that the Selden application discusses--DNA of retroviral origin can disrupt an important gene or regulatory sequence of the host because the DNA can integrate almost anywhere:

> In an ideal world, the introduced gene would be targeted to replace precisely the defective one by site-specific recombination. This would not only eliminate **the danger inherent in random insertion**; it would also guarantee correctly regulated gene expression--a truly major advance.

(Ex. 3 at 214, col. 1; emphasis added).

17. The Selden application next states the most important problem with DNA of retroviral origin in gene delivery systems:

> Third, as recombination between the replication-deficient retroviruses utilized for infection and the endogenous retroviruses present in mammalian genomes is known to

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> occur (Hock, R.A., et al., Nature 320:275-277 (1986)), there is the potential of initiating a chronic retroviral infection in the host animal.

(Ex. 4 at 4, line 32, through 5, line 4).

18. Many species of animals, including humans, have retroviral sequences ("endogenous retroviruses") integrated into their genomes. These proviral sequences are often harmless. However, their activation has been implicated in some cancers. Since exogenous DNA of retroviral origin can recombine with the nucleic acid of the endogenous retroviruses, it can complement or activate the proviral sequences, perhaps resulting in a cancer cell. Furthermore, the recombination of the exogenous DNA of retroviral origin with the proviral sequences can create a new retrovirus capable of infecting the host. (Ex. 2 at 407, col. 1-2).

19. The Robertson article also discusses this third problem with retroviruses:

> The helper virus in the cultures was an unpleasant surprise: it materialized through recombination between the packaging-defective mutant and the vector during co-incubation, because of a short region of homology between the vector and helper viral sequences . . . . This rather strikingly illustrates some of the hazards of working with retroviral vectors--people do worry about the possibility of recombination between vectors and endogenous viral sequences in human cells . . . .

(Ex. 3 at 213, col. 3).

20. In light of the belief of those skilled in the art that methods using retroviral vectors were the best methods for *ex vivo* gene therapy, and their belief that the known problems with retroviral vectors could be overcome, one skilled in the art who read the Selden application in 1987 would have recognized that Dr. Selden invented a composition and a method for transferring a gene into an animal that did not use a retroviral vector or DNA of retroviral origin.

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21. My conclusion that Dr. Selden invented a composition and a method for transferring a gene into an animal that did not use a retroviral vector or DNA of retroviral origin would have been further evident from the Selden application's discussion of particular promoters at pages 18-19. One reason many in the field focused upon retroviral vectors was because their own regulatory elements could be used easily. (Ex. 2 at 406, col. 1). The Selden application does not discuss a single retroviral promoter. Rather, the Selden application discusses endogenous promoters from mammals, fruit flies, and yeast. This is striking because retroviral promoters were widely used by those skilled in the art. The absence of any discussion of retroviral promoters, combined with the discussion of the disadvantages and dangers of retroviral vectors and DNA of retroviral origin, would have conveyed to one skilled in the art that Dr. Selden invented a composition and a method of transferring a gene into an animal that does not involve a retroviral vector or DNA of retroviral origin.

22. In addition to discussing problems with using retroviral vectors, in particular, for *ex vivo* gene therapy, the Selden application also discusses problems with using viral vectors, in general, for *ex vivo* gene therapy:

> At present, however, no single technique appears to be wholly satisfactory. The use of viral vectors suffers from their potential for rearrangement of endogenous genes, as well as their potential for inducing carcinogenesis.

(Ex. 4 at 6, lines 27-31).

23. Given the Selden application's discussion of the disadvantages and dangers of viral vector gene delivery systems, especially retroviral vector gene delivery systems, and DNA of retroviral origin, the specification then goes on to describe a solution to these problems, which does not involve the use of viral vectors, retroviral

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vectors, or DNA of retroviral origin. Specifically, the Selden application describes transfecting somatic cells without a viral vector, without a retroviral vector, and with no DNA of retroviral origin; screening the transfected somatic cells; cloning and expanding a selected somatic cell; and administering the resulting cloned and expanded cells to the recipient subject. (See, e.g., ex. 4 at 12, line 22, through 13, line 6). By not using a viral vector, by not using a retroviral vector, by not using DNA of retroviral origin, by selecting the transfected cells, and by cloning and expanding the selected cells, the Selden application avoids these disadvantages and dangers of viral vectors, retroviral vectors, and DNA of retroviral origin.

24. The nonviral aspects of Dr. Selden's invention are further evident from the experiments reported in the Selden application. At a time when workers in field of *ex vivo* gene transfer and gene therapy research had rejected nonviral methods as inherently too inefficient to be useful, and at a time when these workers had turned to the well characterized and easily manipulatable retroviral vectors, none of the experiments the Selden application reports in its 21-pages of examples uses a viral vector, none of the experiments uses a retroviral vector, and none of the experiments uses DNA of retroviral origin.

25. For all these reasons, it is my opinion the Selden application would have conveyed to one skilled in the art that Dr. Selden invented a composition and a method of transferring a gene into an animal for *ex vivo* gene therapy that did not require the use of a viral vector, a retroviral vector, or DNA of retroviral origin.

26. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true.

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I further declare that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and with the knowledge that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

June 17 1998

Date: ~~May~~ __, 1998

Howard M. Goodman

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